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(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Recombinant Herpes Viruses, a Vaccine Based on These Recombinants, Their Preparation Process, Genes, Vectors and Plasmids Used in This Process

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New recombinant herpes viruses, a vaccine based on these recombinants, their preparation process, genes, vectors and plasmids used in this process.

Abstract

The invention concerns the sequence of the unique short region Us of Marek's disease virus coding for the kinase protein. It also concerns the recombinant herpes viruses in which a heterologous gene has been inserted in the homologous region of the gene coding for the kinase protein and, in particular, the Marek recombinant viruses expressing a gene of an avian pathogenic agent which could possibly be a gene of another serotype of the Marek viruses. It also concerns a process for the preparation of these recombinants as well as the vaccines obtained.

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New recombinant herpes viruses, a vaccine based on these recombinants, their preparation process, genes, vectors and plasmids used in this process.

The present invention concerns recombinant herpes viruses, in particular, for producing vaccines, their process of preparation and the plasmids produced during this process. Moreover, it concerns a part of the chromosome of Marek's disease virus (MDV) which can be used for preparing such vaccines.

Different types of viruses have been used as expression vectors of foreign genes, in particular of genes coding for antigenic proteins, and have proven their potential for immunizing animals. The vaccinia virus has, to a great extent, been used for constructing recombinant viruses. The herpes viruses have also been used: the herpes simplex virus (HSV) (M. Shih et al., Proc. Natl. Acad. Sci., USA. 1984, 81, 5867-5870), the varicella virus (VZV) (R. Lowe et al., Proc. Natl. Acad. Sci., USA. 1987, 84, 3896-3900). The foreign gene is inserted into a fragment of the genomic DNA of the herpes virus, corresponding to a non-essential region for the viral replication, cloned in a plasmid. This gene is transferred into the viral genome by homologous recombination. This latter step is carried out by cotransfection of the herpes genomic DNA and plasmid since this genomic DNA is by nature infectious.

Different genes of herpes viruses have been identified as non-essential to viral growth, certain of these genes being associated with virulence.

- The gene of the thymidine kinase of the herpes simplex virus (D. Dubbs et al., Virology, 1965, 22, 493-502), of the Aujeszky virus (G. Tatarov, Zentralbl. Vet. Med., 1968, 15 B, 848853), of the rhinotracheal infectious bovine virus (S. Kit et al., Virology, 1983, 130, 381-389).
- The gene gIII of the Aujeszky virus (A. Robins et al., J. Virol., 1986, 59, 635-645).
- The gene gX of the Aujeszky virus (D. Thomsen et al., J. Virol., 1987, 61, 229-232).
- The gene gI of the Aujeszky virus (C. Mettenleiter et al., J. Virol., 1987, 61, 4030-4032).

Viruses in which one or other of these genes has been deleted nonetheless retain the capacity to produce a latent infection in mice.

Studies pertaining to the unique short region of the genome of the herpes simplex virus HSV-1 have been conducted (B. Megnier et al., Virology, 1988, 162, 251-254) and have shown that the viruses HSV-1 in the short region of which a gene has been deleted, have undergone an attenuation.

For their part, F.C. Purves et al. (Journal of Virology, 1987, vol. 61, No. 9, 2896-2901) have demonstrated that the open reading frame US3 of the short fragment of the HSV-1 virus genome codes for a virus enzyme, the kinase protein, and is not essential to the replication of said virus.

Studies have been undertaken on Marek's disease virus which belongs to the subfamily of gamma herpes viruses. This is an enveloped virus having a double stranded linear genomic DNA of

about 175 kilobases. Its genome is composed of a long segment (UL) and of a short segment (Us) fram d by r peated inversed terminal sequences.

Marek's disease virus causes paralysis and a lymphoproliferative disease in chickens, usually, at the age of 2 to 5 months. This disease results in very significant economic losses (L. Payne, Biology of Marek's Disease Virus and the Herpes Virus of Turkeys, in The Herpes Virus, vol. 1, pp. 347-431, edited by B. Roizman, Plenum Press).

The strains of Marek's disease virus have been classified into three serotypes:

- serotype 1 comprises the pathogenic strains and attenuated strains derived therefrom.
- serotype 2 comprises the naturally attenuated strains.
- serotype 3 comprises the herpes virus of turkeys (HVT) and its variants.

Consequently, the term Marek's attenuated disease virus will designate serotypes 1, 2 and 3 at the same time.

Marek's disease virus (MDV) and herpes virus of turkeys (HVT) have similar genomic arrangements (A. Buckmaster et al., J. Gen. Virol., 1988, 69, 2033-2042) and numerous homologies of sequence all along their genome (C. Gibbs et al., Proc. Acad. Natl. Sci. USA, 1984, 81, 3365-3369).

The chicks are vaccinated at the age of one day and are then protected against Marek's disease for their entire life. For numerous years, vaccination with the herpes virus of live turkeys (HVT) has been very effective for controlling the disease.

Nevertheless. the emergence of new viral strains which are highly virulent has led to the use of strains of attenuated Marek's disease viruses of another serotype, to vaccinate and thus increase the level of protection, either, for example, the strain CVI 988, MDV serotype 1 attenuated by passing over cells (B. Rispens et al., Avian Dis., 1972, 16, 1108-125), or, for example, the association of the HVT MDV serotype 3 and SB1 strains, MDV serotype 2 (K. Schat et al., J. Natl. Cancer Inst., 1987, 60, 1075-1082).

The genome of Marek's virus has certain similarities with the genome of alpha viruses, herpes simplex (HSV) and chickenpox (VZV). Most of the genes localized in the long region UL of the genome are approximately colinear between the herpes simplex virus, chickenpox (D. McGeogh, J. Gen. Virol., 1988, 69, 1531-1574) and Marek's disease virus (A. Buckmaster, 1988). Thus. in the international patent application WO 90/02802, it was proposed that the genes be inserted into this UL region of HVT and MDV.

On the other hand, the localization of genes in the Us segment shows a larger divergence between herpes viruses. Also among the dozen open reading frames identified for the herpes simplex virus, only four have a homology with the chickenpox virus (McGeogh, 1988, cited above).

In fact, the prior art does not suggest that there is an interest in proceeding with a homology study of open reading frames of the unique short region of the genome of Marek's disease virus with the HSV-1 genes.

The present invention divulges, for the first time, the sequence coding for the kinase protein of the genome of Marek's disease virus (MDV) and allows one to establish, in a surprising manner, that this gene can be deleted without blocking the viral replication and allowing the insertion of heterologous sequences opening the way for development of a series of viral expression vectors.

This type of a recombinant and attenuated virus of Marek's disease constitutes a choice candidate for developing a viral vector expressing foreign genes to be used for polyvalent vaccination of poultry since it has the advantage of being able to be used for its own vaccinal properties and as a vaccine against other viral, bacterial and parasitic diseases as, for example, infectious avian bronchitis, Newcastle's disease, fowl plague, egg-drop syndrome, Gumboro's disease, chicken anaemia, coccidiosis, fowl pox, infectious laryngotracheitis, avian colibacillos, is pasteurellosis, haemophilosis.

SUMMARY OF THE INVENTION

The object of the invention is to provide recombinant herpes viruses, including a recombinant attenuated Marek's disease virus (serotypes 1, 2 or 3), recombinant which can be used as a vaccine, the method for constructing such a recombinant virus as well as a vector virus allowing the multivalent vaccination against viral, bacterial or parasitic avian diseases.

One object of the invention is the nucleotide sequence

and its variants corresponding to the US3 gene which is homologous to the kinase protein gene of the herpes simplex virus and the surrounding regions. The term variants of the nucleotide sequence, as commonly used, means any equivalent sequence such as obtained, for example, by degeneration of the code, minor modifications, mutations or corresponding to viral variants.

Another object of the invention is a recombinant virus selected from the herpes viruses, including the viruses of pseudorabies disease, infectious bovine rhinotracheitis, equine rhinopneumonitis, feline rhinotracheitis, canine herpes.

Another object of the invention is also a Marek's disease recombinant virus comprising one or more heterologous genes inserted in the region of its genome corresponding to US3 gene in such a way so as to be expressed.

By "heterologous gene", one means, in particular, a gene coding for a protein or an immunogenic glycoprotein of a viral, bacterial or parasitic pathogenic agent, in particular, an agent associated with an avian pathology. This also relates to the construction of hybrid viruses, for example, by introducing, into the genome of a turkey herpes virus, genes coding for immunogenes of a Marek's disease virus of serotype 1 and/or serotype 2.

"Heterologous gene" is also intended to mean a gene coding for a peptide or a protein, for example, hormone, growth factor, immunomodulator.

The heterologous gene is preferably expressed under the control of regulating sequences of US3 gene transcription. One can, however, see to it that this expression is either controlled

by a promoting sequence coming from another gene of the virus in question, for example, the promoter of TK gene, of gA gene, of gB gene or from another herpes virus, for example, the promoter of gI gene of the infectious bovine rhinotracheitis virus or from gene II or gene III of pseudorabies virus.

Preferably, the start and stop codons of US3 gene are substituted by those of the gene to be expressed.

DESCRIPTION OF THE FIGURES

Figure 1 shows the construction of the plasmid pMDV 53L which contains the lacZ gene inserted instead of US3 gene.

Figure 2 shows the construction of the plasmid pMDV 53 CL which contains the lacZ gene under the control of the promoter iE of the human cytomegalovirus instead of US3 gene.

Figure 3 shows the plasmid pMDV 53F which contains the gene of the fusion protein of Newcastle's disease virus instead of US3 gene.

DESCRIPTION OF THE INVENTION: MATERIALS AND METHODS

Viral Strain

The serotype 1 strain RB1B of Marek's disease virus (MDV) was used (Schat K.A. et al., 1982, Avian Pathol. 11, 593-605).

The virus culture methods and methods for extraction of viral DNA having a high molecular weight have been described (C. Lee et al., 1980, J. Gen. Virol., 51, 235-253; N. Ross et al., 1989, 70, 1789-1804).

Cell Culture

The fibroblasts of chicken embryo (CEP) were cultivated in 199 F10, medium supplemented by penicillin, streptomycin, fungizone and fetal calf serum, as described (N. Ross, 1975, J. Gen. Virol., 28, 37-47).

Cloning of Viral DNA

Generally, the techniques used for the construction of recombinant plasmids are those described by T. Maniatis et al. (T. Maniatis et al., 1982, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor. NY).

For all cloning and subcloning steps, the vector linearized by the appropriate restriction enzymes is dephosphorized before ligation. The purification of the DNA fragments starting from an agarose gel is done according to the technique described by the manufacturer: "Geneclean" (Bio 101, San Diego, California, USA).

Sequencing

The cloned fragments are sequenced according to the classic technique described by Sanger (G. Sanger, S. Nicklen. A. Coulson, 1977, Proc. Natl. Acad., USA 74, 5463-5467). The sequences after translation have been compared to the published sequences of herpes simplex virus and chickenpox (McGeogh, 1985, J. Mol. Biol. 181, 1, 13; K. Davison et al., 1986, J. Gen. Virol. 67, 1759-1816).

Controlled Mutagenesis

The DNA fragments, subcloned in the Blue Script vector (Stratagene, La Jolla, California, USA) are mutagenesized after

separation of the DNAs simple fragment with the help of the R408 helper phage (Stratagene, La Jolla, California, USA) (M. Russel, S. Kidd. M. Kelley, 1986, Gene 45, 333-338).

The mutagenesis procedure and selection of the mutants by using the strain CJ 236 dut-, ung- of E. coli (In Vitrogen, San Diego, California, USA) is described by T. Kunkel, (T. Kunkel 1985, Proc. Natl. Acad. Sci., 82, 488-492 and T. Kunkel et al., 1987, Methods of Enzymology 154, 367-382, Acad. Press).

In vivo Recombination

The recombinant viruses are obtained according to the conventional techniques of transfection of the sensitive cells, such as the calcium phosphate method or the one using the Lipofectine reactive described by the manufacturer BRL (P.L. Felgner et al., 1987. Proc. Natl. Acad. Sci., USA, 84, 7413). For this, the chicken embryo fibroblasts, cultivated to confluence, are cotransfected with the genomic DNA and the plasmid carrier of the DNA fragment to be inserted, flanked in 5' and 3' by the genome sequences which allows the recombination.

The recombinant viruses can then be screened by hybridization with an appropriate probe or by plaque colouring. When a gene marker, the lacZ gene of β-galactosidase, is inserted into the gene of Marek's disease virus, the expression of this gene can be followed by adding, to the cell covering, an agarose overcoat enclosing the chromogenic substrate for the β-galactosidase, e.g. Xgal (5-bromo-4-chloro-3-indolyl, B.D. galactopyranoside).

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Example 1: Isolating an EcoRI fragment of 5.25 kilobases.

The viral genomic DNA was digested by the restriction enzyme EcoRI and the fragments cloned in the vector pUC 13 (Pharmacia) (Yannisch, Perron et al., 1985, Gene 33, 103-119).

Among the cloned fragments, a fragment of 5.25 kilobases, localized at the level of the small fragment Us, has been, more particularly, analyzed by sequencing (pMDV 05; sequence ID no. 1).

The sequence comprises six open reading frames (ORF). The translated sequences of 4 of these ORF have a homology with the type I HSV virus proteins, localized in the Us fragment. In particular, the US3 gene of Marek's disease virus has a homology with the gene of the kinase protein of the herpes simplex virus.

Surprisingly, the study of this region has shown that the US3 gene can be deleted without blocking the viral replication.

Example 2: Construction of a plasmid pMDV 53L for which the US3 gene has been replaced by the lacZ gene (Figure 1).

The EcoRI fragment of 5.25 kilobases stemming from the clone pMDV 05 was digested by the NcoI enzyme and the extremities thus generated restored by the DNA polymerase Klenow fragment. It was then digested by the KpnI enzyme and the fragment of 1989 pairs of bases thus liberated was cloned in the Blue Script vector linearized by the enzymes EcoRV and KpnI to give the plasmid pMDV

52 having 4947 pairs of bases.

The NcoI and SalI sites were respectively introduced to the extremities 5' and 3' of the cloned fragment by controlled mutagenesis using the oligonucleotides designated by Seq ID no. 2 and 3, in the list of attached sequences, which generates the pMDV 53 plasmid. The lacZ gene was purified from the pMC 1871 plasmid (Pharmacia LKB, Uppsala, Sweden) (S.K. Shamira et al. 1983, Gene 25, 71-82) by digestion by the enzymes SmaI and SalI.

It was then inserted into the pMDV 53 plasmid, partially digested by the NcoI enzyme, treated by the DNA polymerase Klenow fragment and then controlled by the enzyme SalI, which generates the plasmide pMDV 53L of about 6687 pairs of bases.

Example 3: Preparation of a Marek's disease virus comprising the lacZ gene.

The chicken embryo fibroblasts were cotransfected with the total chromosomal DNA of the virus and the DNA of the linearized plasmid pMDV 53L (10 to 50 μg). The cultures were observed for 4 to 6 days until infectious centres appeared. Alternatively, the cells were trypsinated after 72 hours, then reinoculated (1:1 or 1:2) in a secondary passage, until lysis segments were obtained.

The medium was then replaced by the new medium comprising 1% agarose and 0.5% Xgal.

The plaques which are due to the recombination viruses are distinguished by their blue colour.

The viruses can thus be purified by plaque purification and, after inoculation with healthy cells, give cytopathogenic effect shapes coloured in blue in the presence of Xgal.

Example 4: Construction of the plasmid pMDV 53 CL which comprises the lacZ gene under the control of the immediate early promoter of the human cytomegalovirus (CMV) (Figure 2).

The lacZ gene of β-galactosidase was placed under the control of the immediate early promoter of the human cytomegalovirus (IECMV) in the vector pCMV-lacZ.

The fragment of about 4500 pairs of bases comprising the whole, the promoter IE of the cytomegalovirus and the lacZ gene, were digested by the EcoRI enzyme and the extremities filled by the DNA polymerase Klenow fragment. It was then digested by the SalI enzyme.

The fragment thus liberated was cloned in the partially digested pMDV 53 vector, by the NcoI enzyme, treated by the DNA polymerase Klenow fragment and then digested by the SalI enzyme. This plasmid composed of about 8200 pairs of bases is called pMDV 53 CL.

Example 5: Construction of a Marek recombinant virus for which the lacZ gene was introduced under control of the immediate early promoter of the cytomegalovirus, instead of the US3 gene.

The CEP were cotransfected with the genomic DNA of the virus and from 10 to 50 μg of DNA of the linearized plasmid pMDV 53 CL.

One can thus obtain the recombinant viruses which are distinguished by the appearance of blue-coloured infectious plaques in the presence of the chromogen substrate Xgal. These viruses were purified according to the plaque purification technique and allowed to infect the secondary chicken embryo fibroblasts.

The blue-coloured infectious plaques can be obtained in the presence of Xgal which shows that the lacZ gene is inserted at the locus of the US3 gene.

Example 6: Construction of the pMDV 53F plasmid which comprises the gene of the fusion protein of Newcastle's disease virus instead of the US3 gene (Figure 3).

The fusion gene (J. Taylor et al., 1990, J. Virol., 64, 1441-1450) was introduced in the form of a fragment at the blunt ends in the Blue Script vector at the SmaI site to give the pNF1 plasmid having 5300 pairs of bases.

NcoI and SalI sites were introduced by controlled mutagenesis at the level of the ATG and stop codons of the fusion gene, due to the oligonucleotides indexed SEQ ID no. 4 and SEQ ID no. 5 respectively in the list of the attached sequences.

The fragment of 1682 pairs of bases NcoI/SalI coming from the pNF2 plasmid was inserted into the pMDV 53 vector partially digested by the NcoI enzyme and digested by the SalI enzyme to give the pMDV 53F plasmid having 5369 bases pairs.

Example 7: Construction of a Marek's disease virus comprising the fusion protein gene of Newcastle's disease virus.

The chicken embryo fibroblasts were cotransfected with the total genomic DNA of the virus and 10 to 50 µg of linearized DNA of the pMDV 53F plasmid. The cultures were observed for the appearance of infectious plaques.

The recombinant viruses were then screened by hybridization with a probe including the fusion gene.

Similar procedures were used for the construction of the non-avian recombinant herpes viruses, by inserting a heterologous gene in the Us region and, in particular, in the homologous gene at US3 gene of Marek's virus.

The invention also concerns vaccines, live or not, made up of or containing recombinant viruses constructed according to the invention, or containing immunogenes expressed by these viruses.

APPENDIX I

LIST OF SEQUENCES

SEQ ID no. 1

Length of sequence: 5.255 pairs of bases

Type of molecule sequenced: genomic DNA

Origin of the molecule: Marek's disease virus, strain RB1 B

Experimental source: pMDV 05 plasmid

Characteristics:

from	1	to 324	pairs of bases:	non-coding region
from	325	to 1,135	pairs of bases:	US1 gene. The gene is coded by the complementary DNA segment at the indicated sequence and is transcribed from right to left (SEQ ID no. 1.B)
from	623	to 1,214	pairs of bases:	US2 gene
from	1,215	to 1,245	pairs of bases:	non-coding region
from	1,246	to 2,451	pairs of bases:	US3 gene
from	2,452	to 2,563	pairs of bases:	non-coding region
from	2,564	to 3,004	pairs of bases:	US4 gene
from	3,005	to 3,190	pairs of bases:	non-coding region
from	3,191	to 4,384	pairs of bases:	US5 gene
from	4,385	to 4,494	pairs of bases:	non-coding region
from	4,495	to 5,253	pairs of bases:	US6 gene

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CAAAAATTTACATTAGTAATCTTTCTCGGTGGCTTACCAAATCGTCCTCTTGGTATATCCATATCATCGAAC   72

ATTGTAGCATTGACTCTGCTCATCGTTGTCTTTCAAATGCGCTCGATTGTTGAATCTCTCCTGATGTTAGAA  144

GTATATGGAAGATAGCCTGGATACATAAGTGATCTAGAAGGGTTTGTTATTGCAGTAATATACAAATTATAC  216

GTGACACTATAGCGACGGTTGTAGCGATGCACCTAATCGTAATGTGTATACGCCCCATCATGTAATTATATC  288

TAATTGGTAGCAAGTAGGTCTGTCGAATAACAGCTAATGACTACCGGCTCTACATTTTTTCTGTATTCGTGA  360

CTTTCCTGTCGCAGTGTAACGAACCGGAATTGCAATCGCATCTCTATCTTCTTTCTTGCAACATTTTCCACA  432

ACAGAATAATCTGCCGGGTGTACTACTCATTTGAGGTGGTTCGATTTCCGGAGGTTTTAGAGGATTGGGTGG  504

GGACCCGAGGATTTTGTATACACATACCATATCACTGTCGCAAAAATGCGCTCTATCTTCTGGGGTGTCGAA  576

CTTCGGTTCCCATGTAGATGTCAAGAGAGTTTGAATATTGTCGGGA ATG GCC CAC GGC ATA CCG     640
                                                Met Ala His Gly Ile Pro       6

GAC CAG GTC CCA GAC ACT TTG ATT GCA AGT AAC CTT TTT GGC AAA GGA ATA CAT     694
Asp Gln Val Pro Asp Thr Leu Ile Ala Ser Asn Leu Phe Gly Lys Gly Ile His      24

TCG AGC GCA ATG CGA CAT ATA TCT GCC GCC CCA ACT ATC CAC AAG CTA TGT GGA     748
Ser Ser Ala Met Arg His Ile Ser Ala Ala Pro Thr Ile His Lys Leu Cys Gly      42

GCA TTA CCA GAA ACT TCA GAT TCC AAC ATC AAA TAT CCA GAT AGA ACA TCC TGC     802
Ala Leu Pro Glu Thr Ser Asp Ser Asn Ile Lys Tyr Pro Asp Arg Thr Ser Cys      60

CAT TCT GTG GAA CAT CCT GCA ACA TCT TCA AAT AGC CGC ACT ATA AAC GAA TCC     856
His Ser Val Glu His Pro Ala Thr Ser Ser Asn Ser Arg Thr Ile Asn Glu Ser      78

CTA GTT CCG GCC AAT CCG GTA CCA CGA ACT CCA GTT CCA TCT GGT GGC TTT GTC     910
Leu Val Pro Ala Asn Pro Val Pro Arg Thr Pro Val Pro Ser Gly Gly Phe Val      96

CTT ACT ATC GGT CGA TGT TGC CGA GGA AGA ATT AAC ATG GGT TTG GCA AAA CGG     964
Leu Thr Ile Gly Arg Cys Cys Arg Gly Arg Ile Asn Met Gly Leu Ala Lys Arg     114

AAT AGG TCT GCA GCT CTG ACG ATT ATG GGC ACA CCC ACA TCA TCC TGT ATT TGT    1018
Asn Arg Ser Ala Ala Leu Thr Ile Met Gly Thr Pro Thr Ser Ser Cys Ile Cys     132

TCC ATA CAT TGC TTT ATA AGG AAT ATC CAT AAA GTA GAT GCA GCA TCT CTA GAT    1072
Ser Ile His Cys Phe Ile Arg Asn Ile His Lys Val Asp Ala Ala Ser Leu Asp     150

CTT CCT GGC AAT CGA TCG CAT TCA TCT AGA AGT GTG ACT ATA GTT ATC ATG GAC    1126
Leu Pro Gly Asn Arg Ser His Ser Ser Arg Ser Val Thr Ile Val Ile Met Asp     168

ACA CCC ATC TTC ACT CCA CCA ATA ATC TTT TTT ATT GTT AAT AAC TGG GCC GGT    1180
Thr Pro Ile Phe Thr Pro Pro Ile Ile Phe Phe Ile Val Asn Asn Trp Ala Gly     186

CTG ATC TCC AAA TCT TAT ACC TCT GGT AGA ATA  TGAAACAGGGTTAAAACTAGGTAATAG   1240
Leu Ile Ser Lys Ser Tyr Thr Ser Gly Arg Ile                                 197

ACTGGATG TCT TCG AGT CCG GAG GCA GAA ACG ATG GAA TGC GGC ATT TCT TCG TCG    1296
     Met Ser Ser Ser Pro Glu Ala Glu Thr Met Glu Cys Gly Ile Ser Ser Ser     214

AAA GTA CAC GAC TCT AAA ACT AAT ACT ACC TAC GGA ATT ATA CAT AAC AGC ATC    1350
Lys Val His Asp Ser Lys Thr Asn Thr Thr Tyr Gly Ile Ile His Asn Ser Ile     232

AAT GGT ACG GAT ACG ACG TTG TTT GAT ACT TTT CCC GAC AGT ACC GAT AAC GCG    1404
Asn Gly Thr Asp Thr Thr Leu Phe Asp Thr Phe Pro Asp Ser Thr Asp Asn Ala     250

GAA GTG ACG GGG GAT GTG GAC GAT GTG AAG ACT GAG AGC TCT CCC GAG TCC CAA    1458
Glu Val Thr Gly Asp Val Asp Asp Val Lys Thr Glu Ser Ser Pro Glu Ser Gln     268
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TCT GAA GAT TTG TCA CCT TTT GGG AAC GAT GGA AAT GAA TCC CCC GAA ACG GTG  1512
Ser Glu Asp Leu Ser Pro Phe Gly Asn Asp Gly Asn Glu Ser Pro Glu Thr Val   286

ACG GAC ATT GAT GCA GTT TCA GCT GTG CGA ATG CAG TAT AAC AAT GTT TCA TCG  1566
Thr Asp Ile Asp Ala Val Ser Ala Val Arg Met Gln Tyr Asn Asn Val Ser Ser   304

TTA TCG CCC GGA TCT GAA GGG TAT ATC TAT GTT TGT ACA AAG CGT GGG GAT AAT  1620
Leu Ser Pro Gly Ser Glu Gly Tyr Ile Tyr Val Cys Thr Lys Arg Gly Asp Asn   322

ACC AAG AGA AAA GTC ATT GTG AAA GCT GTG ACT GGT GAC AAA ACC CTT GGG AGT  1674
Thr Lys Arg Lys Val Ile Val Lys Ala Val Thr Gly Asp Lys Thr Leu Gly Ser   340

GAA ATT GAT ATA TTA AAA AAA ATG TCT CAC CGC TCC ATA ATT AGA TTA GTT CAT  1728
Glu Ile Asp Ile Leu Lys Lys Met Ser His Arg Ser Ile Ile Arg Leu Val His   358

GCT TAT AGA TGG AAA TCG ACA GTT TGT ATG GTA ATG CCT AAA TAC AAA TGC GAC  1782
Ala Tyr Arg Trp Lys Ser Thr Val Cys Met Val Met Pro Lys Tyr Lys Cys Asp   376

TTG TTT ACG TAC ATA GAT ATC ATG GGA CCA TTG CCA CTA AAT CAA ATA ATT ACG  1836
Leu Phe Thr Tyr Ile Asp Ile Met Gly Pro Leu Pro Leu Asn Gln Ile Ile Thr   394

ATA GAA CGG GGT TTG CTT GGA GCA TTG GCA TAT ATC CAC GAA AAG GGT ATA ATA  1890
Ile Glu Arg Gly Leu Leu Gly Ala Leu Ala Tyr Ile His Glu Lys Gly Ile Ile   412

CAT CGT GAT GTA AAA ACT GAA AAT ATA TTT TTG GAC AAA CCT GAA AAT GTA GTA  1944
His Arg Asp Val Lys Thr Glu Asn Ile Phe Leu Asp Lys Pro Glu Asn Val Val   430

TTG GGG GAC TTT GGG GCA GCA TGT AAA TTA GAT GAA CAT ACA GAT AAA CCC AAA  1998
Leu Gly Asp Phe Gly Ala Ala Cys Lys Leu Asp Glu His Thr Asp Lys Pro Lys   448

TGT TAT GGA TGG AGT GGA ACT CTG GAA ACC AAT TCG CCT GAA CTG CTT GCA CTT  2052
Cys Tyr Gly Trp Ser Gly Thr Leu Glu Thr Asn Ser Pro Glu Leu Leu Ala Leu   466

GAT CCA TAC TGT ACA AAA ACT GAT ATA TGG AGT GCA GGA TTA GTT CTG TTT GAG  2106
Asp Pro Tyr Cys Thr Lys Thr Asp Ile Trp Ser Ala Gly Leu Val Leu Phe Glu   484

ATG TCA GTA AAA AAT ATA ACC TTT TTT GGC AAA CAA GTA AAC GGC TCA GGT TCT  2160
Met Ser Val Lys Asn Ile Thr Phe Phe Gly Lys Gln Val Asn Gly Ser Gly Ser   502

CAG CTG AGA TCC ATA ATT AGA TGC CTG CAA GTC CAT CCG TTG GAA TTT CCA CAG  2214
Gln Leu Arg Ser Ile Ile Arg Cys Leu Gln Val His Pro Leu Glu Phe Pro Gln   520

AAC AAT TCT ACA AAC TTA TGC AAA CAC TTC AAG CAG TAC GCG ATT CAG TTA CGA  2268
Asn Asn Ser Thr Asn Leu Cys Lys His Phe Lys Gln Tyr Ala Ile Gln Leu Arg   538

CAT CCA TAT GCA ATC CCT CAG ATT ATA CGA AAG AGT GGT ATG ACG ATG GAT CTT  2322
His Pro Tyr Ala Ile Pro Gln Ile Ile Arg Lys Ser Gly Met Thr Met Asp Leu   556

GAA TAT GCT ATT GCA AAA ATG CTC ACA TTC GAT CAG GAG TTT AGA CCA TCT GCC  2376
Glu Tyr Ala Ile Ala Lys Met Leu Thr Phe Asp Gln Glu Phe Arg Pro Ser Ala   574

CAA GAT ATT TTA ATG TTG CCT CTT TTT ACT AAA GAA CCC GCT GAC GCA TTA TAC  2430
Gln Asp Ile Leu Met Leu Pro Leu Phe Thr Lys Glu Pro Ala Asp Ala Leu Tyr   592

ACG ATA ACT GCC GCT CAT ATG  TAAACACCCGTCAAAAATAACTTCAATGATTCATTTTATAATA 2494
Thr Ile Thr Ala Ala His Met                                               599

TATACTACGCGTTACCTGCAATAATGACAACATTCGAAGTCTTTGAAGATTCGCAGACCTTTTTTGCGAATG 2566
                                                                      Met  600

GCA CCT TCG GGA CCT ACG CCA TAT TCC CAC AGA CCG CAA ATA AAG CAT TAT GGA  2620
Ala Pro Ser Gly Pro Thr Pro Tyr Ser His Arg Pro Gln Ile Lys His Tyr Gly   618

ACA TTT TTG GAT TGC ATG AGA TAT ACT CTA AAC GAT GAG AGT AAG GTA GAT GAT  2674
Thr Phe Leu Asp Cys Met Arg Tyr Thr Leu Asn Asp Glu Ser Lys Val Asp Asp   636
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AGA TGT TCA GAC ATA CAT AAC TCC TTA GCA CAA TCC AAT GTT ACT TCA AGC ATG  2728
Arg Cys Ser Asp Ile His Asn Ser Leu Ala Gln Ser Asn Val Thr Ser Ser Met   654

TCT GTA ATG AAC GAT TCG GAA GAA TAT CCA TTA ATA AAT GGA CCT TCG ATG CAG  2782
Ser Val Met Asn Asp Ser Glu Glu Tyr Pro Leu Ile Asn Gly Pro Ser Met Gln   672

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Ala Glu Asp Pro Lys Ser Val Phe Tyr Lys Val Arg Lys Pro Asp Arg Ser Arg   690

GAT TTT TCA TGG CAA AAT CTG AAC TCC CAT GGC AAT AGT GGT CTA CGT CGT GAA  2890
Asp Phe Ser Trp Gln Asn Leu Asn Ser His Gly Asn Ser Gly Leu Arg Arg Glu   708

AAA TAT ATA CGT TCC TCT AAG AGG CGA TGG AAG AAT CCC GAG ATA TTT AAG GTA  2944
Lys Tyr Ile Arg Ser Ser Lys Arg Arg Trp Lys Asn Pro Glu Ile Phe Lys Val   726

TCT TTG AAA TGT GAA TCA ATT GGC GCT GGT AAC GGA ATA AAA ATT TCA TTC TCA  2998
Ser Leu Lys Cys Glu Ser Ile Gly Ala Gly Asn Gly Ile Lys Ile Ser Phe Ser   744

TTT TTC  TAACATTATAATATATCAGATCGTTTCTTATATACTTATTTTCATCGTCGGGATATGACTAAC 3067
Phe Phe                                                                  746

GTATACTAAGTTACAAGAAACAACTGCTTAACGTCGAACATAACGGAAATAAAAATATATATAGCGTCTCCT 3139

ATAANTGTTATATTGGCACCTTTTAGAGCTTCGGTATGAATAGATACAGATATG AAA GTA TTT TTT  3205
                                                        Met Lys Val Phe Phe   751

TTT AGA TAT ATC TCA TCC ACG AGA ATG ATT CTT ATA ATC TGT CTA CTT TTG GGA  3259
Phe Arg Tyr Ile Ser Ser Thr Arg Met Ile Leu Ile Ile Cys Leu Leu Leu Gly   769

ATT GGG GAC ATG TCC GCA ATG GGA CTT AAG AAA GAC AAT TCT CCG ATC ATT CCC  3313
Ile Gly Asp Met Ser Ala Met Gly Leu Lys Lys Asp Asn Ser Pro Ile Ile Pro   787

ACA TTA CAT CCG AAA GGT AAT GAA AAC CTC CGG GCT ACT CTC AAT GAA TAC AAA  3367
Thr Leu His Pro Lys Gly Asn Glu Asn Leu Arg Ala Thr Leu Asn Glu Tyr Lys   805

ATC CCG TCT CCA CTG TTT GAT ACA CTT GAC AAT TCA TAT GAG ACA AAA CAC GTA  3421
Ile Pro Ser Pro Leu Phe Asp Thr Leu Asp Asn Ser Tyr Glu Thr Lys His Val   823

ATA TAT ACG GAT AAT TGC AGT TTT GCT GTT TTG AAT CCA TTT GGC GAT CCG AAA  3475
Ile Tyr Thr Asp Asn Cys Ser Phe Ala Val Leu Asn Pro Phe Gly Asp Pro Lys   841

TAT ACG CTT CTC AGT TTA CTG TTG ATG GGA CGA CGC AAA TAT GAT GCT CTA GTC  3529
Tyr Thr Leu Leu Ser Leu Leu Leu Met Gly Arg Arg Lys Tyr Asp Ala Leu Val   859

GCA TGG TTT GTC TTG GGC AGA GCA TGT GGG AGA CCA ATT TAT TTA CGT GAA TAT  3583
Ala Trp Phe Val Leu Gly Arg Ala Cys Gly Arg Pro Ile Tyr Leu Arg Glu Tyr   877

GCC AAC TGC TCT ACT AAT GAA CCA TTT GGA ACT TGT AAA TTA AAG TCC CTA GGA  3637
Ala Asn Cys Ser Thr Asn Glu Pro Phe Gly Thr Cys Lys Leu Lys Ser Leu Gly   895

TGG TGG GAT AGA AGA TAT GCA ATG ACG AGT TAT ATC GAT CGA GAT GAA TTG AAA  3691
Trp Trp Asp Arg Arg Tyr Ala Met Thr Ser Tyr Ile Asp Arg Asp Glu Leu Lys   913

TTG ATT ATT GCA GCA CCC AGT CGT GAG CTA AGT GGA TTA TAT ACG CGT TTA ATA  3745
Leu Ile Ile Ala Ala Pro Ser Arg Glu Leu Ser Gly Leu Tyr Thr Arg Leu Ile   931

ATA ATT AAT GGA GAA CCC ATT TCG AGT GAC ATA TTA CTG ACT GTT AAA GAA ACA  3799
Ile Ile Asn Gly Glu Pro Ile Ser Ser Asp Ile Leu Leu Thr Val Lys Glu Thr   949

TGT AGT TTT TCG AGA CGG GGG ATA AAG GAT AAC AAA CTA TGC AAA CCG TTC AGT  3853
Cys Ser Phe Ser Arg Arg Gly Ile Lys Asp Asn Lys Leu Cys Lys Pro Phe Ser   967

TTT TTT GTC AAT GGT ACA ACA CGG CTG TTA GAC ATG GTG GGA ACA GGA ACC CCG  3907
Phe Phe Val Asn Gly Thr Thr Arg Leu Leu Asp Met Val Gly Thr Gly Thr Pro   985

AGA GCT CAT GAA GAA AAT GTG AAG CAG TGG CTT GAA CGA ATT GGT GGT AAA CAT  3961
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Arg Ala His Glu Glu Asn Val Lys Gln Trp Leu Glu Arg Ile Gly Gly Lys His  1003

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Leu Pro Ile Val Val Glu Thr Ser Met Gln Gln Val Ser Asn Leu Pro Arg Ser  1021

TTT AGA GAT TCA TAT TTC AAA TCA CCT GAC GAC GAT AAA TAT GAT GAC GTC AAA  4069
Phe Arg Asp Ser Tyr Phe Lys Ser Pro Asp Asp Asp Lys Tyr Asp Asp Val Lys  1039

ATG ACA TCG GCC ACT ACT AAT AAC ATT ACC ACC TCC GTG GAT GGT TAC ACT GGA  4123
Met Thr Ser Ala Thr Thr Asn Asn Ile Thr Thr Ser Val Asp Gly Tyr Thr Gly  1057

CTC ACT AAT CGG CCC GAG GAC TTT GAG AAA GCA CCA TAC ATA ACT AAA CGA CCG  4177
Leu Thr Asn Arg Pro Glu Asp Phe Glu Lys Ala Pro Tyr Ile Thr Lys Arg Pro  1075

ATA ATC TCT GTC GAG GAG GCA TCC AGT CAA TCA CCT AAA ATA TCA ACA GAA AAA  4231
Ile Ile Ser Val Glu Glu Ala Ser Ser Gln Ser Pro Lys Ile Ser Thr Glu Lys  1093

AAA TCC CGA ACG CAA ATA ATA ATT TCA CTA GTT GTT CTA TGC GTC ATG TTT TGT  4285
Lys Ser Arg Thr Gln Ile Ile Ile Ser Leu Val Val Leu Cys Val Met Phe Cys  1111

TTC ATT GTA ATC GGG TCT GGT ATA TGG ATC CTT CGC AAA CAC CGC AAA ACG GTG  4339
Phe Ile Val Ile Gly Ser Gly Ile Trp Ile Leu Arg Lys His Arg Lys Thr Val  1129

ATG TAT GAT AGA CGT CGT CCA TCA AGA CGG GCA TAT TCC CGC CTA  TAACACGTGTT 4395
Met Tyr Asp Arg Arg Arg Pro Ser Arg Arg Ala Tyr Ser Arg Leu              1144

TGGTATGGGCGTGTCGCTATAGTGCATAAGAAGTTGACTACATTGCATCAATGACATTATATAGCTTCTTTG 4467

GTCAGATAGACGGCGTGTGTGATTGCGATG TAT CTA CTA CAA TTA TTA TTT TGG ATC CGC   4527
                              Met Tyr Leu Leu Gln Leu Leu Phe Trp Ile Arg   1155

CTC TTT CGA GGC ATC TGG TCT ATA GTT TAT ACT GGA ACA TCT GTT ACG TTA TCA  4581
Leu Phe Arg Gly Ile Trp Ser Ile Val Tyr Thr Gly Thr Ser Val Thr Leu Ser  1173

ACG GAC CAA TCT GCT CTT GTT GCG TTC TGC GGA TTA GAT AAA ATG GTG AAT GTA  4635
Thr Asp Gln Ser Ala Leu Val Ala Phe Cys Gly Leu Asp Lys Met Val Asn Val  1191

CGC GGC CAA CTT TTA TTC CTG GGC GAC CAG ACT CGG ACC AGT TCT TAT ACA GGA  4689
Arg Gly Gln Leu Leu Phe Leu Gly Asp Gln Thr Arg Thr Ser Ser Tyr Thr Gly  1209

ACG ACG GAA ATC TTG AAA TGG GAT GAA GAA TAT AAA TGC TAT TCC GTT CTA CAT  4743
Thr Thr Glu Ile Leu Lys Trp Asp Glu Glu Tyr Lys Cys Tyr Ser Val Leu His  1227

GCG ACA TCA TAT ATG GAT TGT CCT GCT ATA GAC GCC ACG GTA TTC AGA GGC TGT  4797
Ala Thr Ser Tyr Met Asp Cys Pro Ala Ile Asp Ala Thr Val Phe Arg Gly Cys  1245

AGA GAC GCT GTG GTA TAT GCT CAA CCT CAT GAT AGA GTA CAA CCT TTT CCC GAA  4851
Arg Asp Ala Val Val Tyr Ala Gln Pro His Asp Arg Val Gln Pro Phe Pro Glu  1263

AAG GGA ACA TTG TTG AGA ATT GTC GAA CCC AGA GTA TCA GAT ACA GGC AGC TAT  4905
Lys Gly Thr Leu Leu Arg Ile Val Glu Pro Arg Val Ser Asp Thr Gly Ser Tyr  1281

TAC ATA CGT GTA GCT CTC GCT GGA AGA AAT ATG AGC GAT ATA TTT AGA ATG GCT  4959
Tyr Ile Arg Val Ala Leu Ala Gly Arg Asn Met Ser Asp Ile Phe Arg Met Ala  1299

GTT ATT ATA AGG AGT AGC AAA TCT TGG GCN TGT AAT CAC TCT GCT AGT TCA TTT  5013
Val Ile Ile Arg Ser Ser Lys Ser Trp  aa Cys Asn His Ser Ala Ser Ser Phe  1317

CAG GCC CAT AAA TGT ATT CGC TAT GTC GAC CGT ATG GCC TTT GAA AAT TAT CTG  5067
Gln Ala His Lys Cys Ile Arg Tyr Val Asp Arg Met Ala Phe Glu Asn Tyr Leu  1335

ATT GGA CAT GTA GGC AAT TTG CTG GAC AGT GAC TCG GAA TTG CAT GCA ATT TAT  5121
Ile Gly His Val Gly Asn Leu Leu Asp Ser Asp Ser Glu Leu His Ala Ile Tyr  1353

AAT ATT ACT CCC CAA TCC ATT TCC ACA GAT ATT AAT ATT ATA ACG ACT CCA TTT  5175
Asn Ile Thr Pro Gln Ser Ile Ser Thr Asp Ile Asn Ile Ile Thr Thr Pro Phe  1371
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Tyr Asp Asn Ser Gly Thr Ile Tyr Ser Pro Thr Val Phe Asn Leu Phe Asn Asn  1389

AAT TCC CAT GTC GAT GCA ATG AAT  TC                                     5255
Asn Ser His Val Asp Ala Met Asn                                         1397
```

SEQ ID no. 1 B

Length of sequence: 1,188 pairs of bases

Type of molecule sequenced: genomic DNA

Origin of the molecule: Marek's disease virus, strain RB1 B

Experimental source: pMDV 05 plasmid

Characteristics:

from	1	to 324	pairs of bases: non-coding region
from	325	to 1,135	pairs of bases: US1 gene: the gene is transcribed from right to left; the indicated sequence is complementary to the sequence SEQ ID no. 1

```
                                                                Met Gly Val Ser
GAGATCAGACCGGCCCAGTTATTAACAATAAAAAAGATTATTGGTGGAGTGAAG ATG GGT GTG TCC  1189

Met Ile Thr Ile Val Thr Leu Leu Asp Glu Cys Asp Arg Leu Pro Gly Arg Ser
ATG ATA ACT ATA GTC ACA CTT CTA GAT GAA TGC GAT CGA TTG CCA GGA AGA TCT 1113

Arg Asp Ala Ala Ser Thr Leu Trp Ile Phe Leu Ile Lys Gln Cys Met Glu Gln
AGA GAT GCT GCA TCT ACT TTA TGG ATA TTC CTT ATA AAG CAA TGT ATG GAA CAA 1069

Ile Gln Asp Asp Val Gly Val Pro Ile Ile Val Arg Ala Ala Asp Leu Phe Arg
ATA CAG GAT GAT GTG GGT GTG CCC ATA ATC GTC AGA GCT GCA GAC CTA TTC CGT 1015

Phe Ala Lys Pro Met Leu Ile Leu Pro Arg Gln His Arg Pro Ile Val Arg Thr
TTT GCC AAA CCC ATG TTA ATT CTT CCT CGG CAA CAT CGA CCG ATA GTA AGG ACA  961

Lys Pro Pro Asp Gly Thr Gly Val Arg Gly Thr Gly Leu Ala Gly Thr Arg Asp
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Asp Val Leu Ser Gly Tyr Leu Met Leu Glu Ser Glu Val Ser Gly Asn Ala Pro
GAT GTT CTA TCT GGA TAT TTG ATG TTG GAA TCT GAA GTT TCT GGT AAT GCT CCA  799

His Ser Leu Trp Ile Val Gly Ala Ala Asp Ile Cys Arg Ile Ala Leu Glu Cys
CAT AGC TTG TGG ATA GTT GGG GCG GCA GAT ATA TGT CGC ATT GCG CTC GAA TGT  745

Ile Pro Leu Pro Lys Arg Leu Leu Ala Ile Lys Val Ser Gly Thr Trp Ser Gly
ATT CCT TTG CCA AAA AGG TTA CTT GCA ATC AAA GTG TCT GGG ACC TGG TCC GGT  691

Met Pro Trp Ala Ile Pro Asp Asn Ile Gln Thr Leu Leu Thr Ser Thr Trp Glu
ATG CCG TGG GCC ATT CCC GAC AAT ATT CAA ACT CTC TTG ACA TCT ACA TGG GAA  637

Pro Lys Phe Asp Thr Pro Glu Asp Arg Ala His Phe Cys Asp Ser Asp Met Val
CCG AAG TTC GAC ACC CCA GAA GAT AGA GCG CAT TTT TGC GAC AGT GAT ATG GTA  583

Cys Val Tyr Lys Ile Leu Gly Ser Pro Pro Asn Pro Leu Lys Pro Pro Glu Ile
TGT GTA TAC AAA ATC CTC GGG TCC CCA CCC AAT CCT CTA AAA CCT CCG GAA ATC  529

Glu Pro Pro Gln Met Ser Ser Thr Pro Gly Arg Leu Phe Cys Cys Gly Lys Cys
GAA CCA CCT CAA ATG AGT AGT ACA CCC GGC AGA TTA TTC TGT TGT GGA AAA TGT  475

Cys Lys Lys Glu Asp Arg Asp Ala Ile Ala Ile Pro Val Arg Tyr Thr Ala Thr
TGC AAG AAA GAA GAT AGA GAT GCG ATT GCA ATT CCG GTT CGT TAC ACT GCG ACA  367

Gly Lys Ser Arg Ile Gln Lys Lys Cys Arg Ala Gly Ser His
GGA AAG TCA CGA ATA CAG AAA AAA TGT AGA GCC GGT AGT CAT  TAGCTGTTATTCGAC  310

AGACCTACTTGCTACCAATTAGATATAATTACATGATGGGGCGTATACACATTACGATTAGGTGCATCGCTA  238

CAACCGTCGCTATAGTGTCACGTATAATTTGTATATTACTGCAATAACAAACCCTTCTAGATCACTTATGTA  166

TCCAGGCTATCTTCCATATACTTCTAACATCAGGAGAGATTCAACAATCGAGCGCATTTGAAAGACAACGAT   94

GAGCAGAGTCAATGCTACAATGTTCGATGATATGGATATACCAAGAGGACGATTTGGTAAGCCACCGAGAAA   22

GATTACTAATGTAAATTTTTG                                                       1
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SEQ ID no. 2

Type of sequence: oligonucleotide

Length of sequence:

Type of molecule: DNA

5' GGA CTC GAA CCA TGG AGT CTA TTA CC 3'

NCO 1

SEQ ID no. 3

Type of sequence: oligonucleotide

Length of sequence:

Type of molecule: DNA

5' GAC GGG TGT CGA CAT ATG AG 3'

SalI

SEQ ID no. 4

Type of sequence: oligonucleotide

Length of sequence:

Type of molecule: DNA

5' CTG GAG CCC ATG GTG CAC CTT TG 3'

NCO1

SEQ ID no. 5

Type of sequence: oligonucleotide

Length of sequence:

Type of molecule: DNA

5' CAA ATT GCT ATT GTC GAC ACC TCC GCC TCT C 3'

SalI

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Nucleotide sequence, and its variants, from the unique short domain (Us) of the virus of Marek's disease, characterized in that it corresponds to the US3 gene, is non-essential to replication, and is homologous with the kinase protein gene of the herpes simplex virus.

2. Nucleotide sequence US3 of the MDV virus, and his variants, according to claim 1 appearing on the sequence ID no. 1.

3. Nucleotide sequence ID no. 1 and genes and their variants including this sequence.

4. Recombinant virus selected from the herpes virus, comprising at least one heterologous gene inserted into the Us region of the genome of said virus corresponding to the gene of the kinase protein.

5. Recombinant virus according to claim 4, characterized in that the gene is a coding gene for a viral, bacterial or parasitic immunogene.

6. Recombinant virus of Marek's disease according to claim 5, comprising at least one heterologous gene, characterized in that this gene is inserted into the region of its genome

corresponding to the US3 gene, in such a way so as to be able to be expressed.

7. Recombinant virus according to claim 6, characterized in that the inserted heterologous gene codes for a pathogen selected from the group consisting of infectious avian bronchitis, Newcastle's disease, Gumboro's disease, fowl plague, chicken anaemia, egg-drop syndrome, fowl pox, infectious laryngo-tracheitis, avian coli bacillosis, pasteurelosis, coccidiosis, haemophilosis.

8. Recombinant virus according to one of claims 6 and 7, characterized in that this is the MDV virus.

9. Recombinant virus according to one of claims 6 and 7, characterized in that this is the HVT virus.

10. Recombinant virus according to claim 9, characterized in that the inserted gene codes for an immunogene of a Marek's disease virus of serotype 1 or 2.

11. Recombinant virus according to any one of claims 4 to 10, characterized in that the gene is inserted in order to be expressed under the control of the transcription regulating sequences of the gene of the kinase protein.

12. Recombinant virus according to one of claims 4 to 11, charact rized in that the heterologous gene inserted is likely to be expressed under the control of promoting sequences of the virus in question, or other herpes viruses.

13. Recombinant virus according to any one of claims 4 to 12, characterized in that the start and stop codons of the gene inserted are substituted for those of the US3 gene.

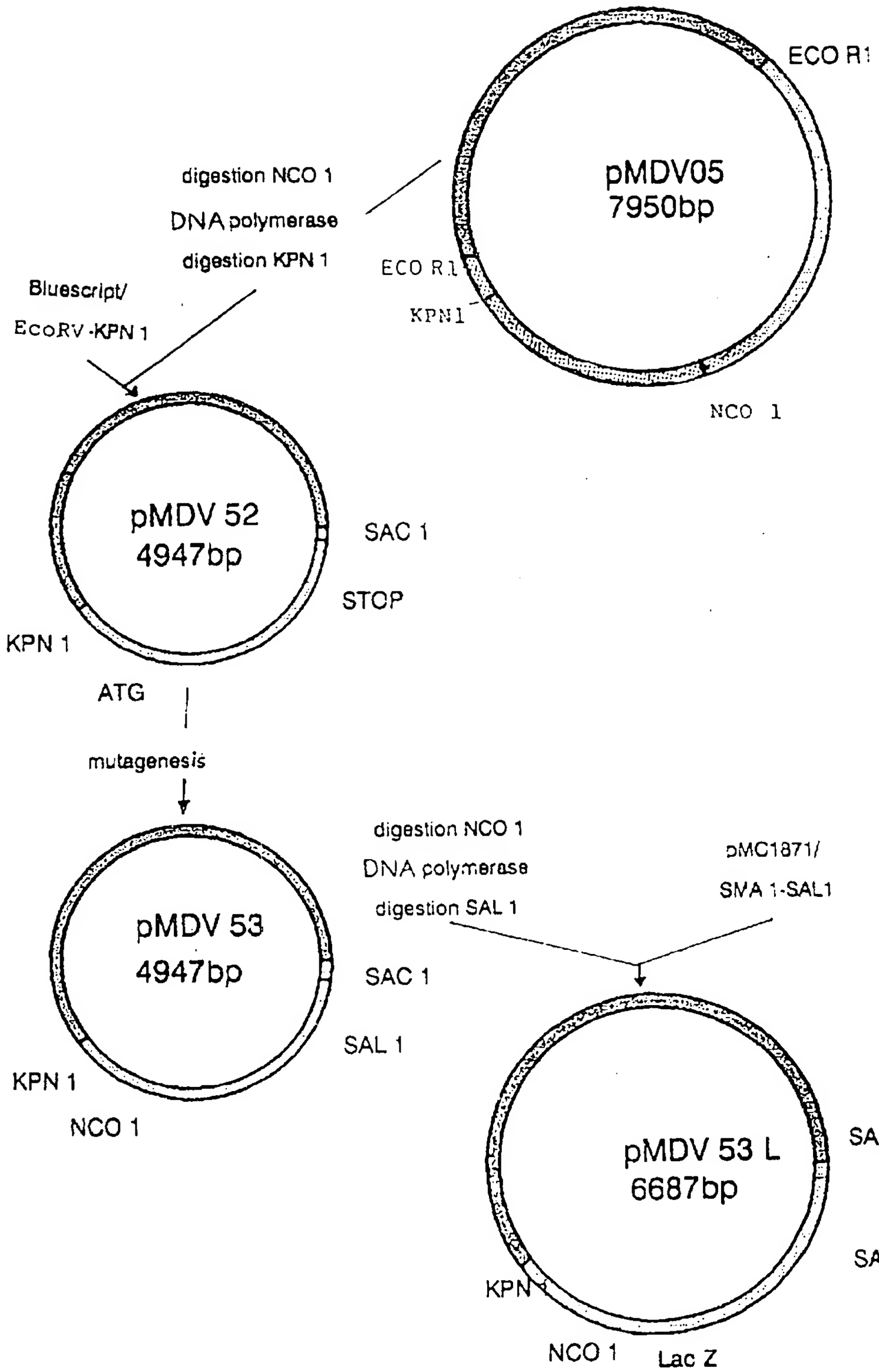
14. Vaccine characterized in that it comprises a recombinant virus according to any one of claims 3 to 9.

15. Process for preparation of a recombinant virus of Marek's disease, characterized in that at least one heterologous gene is inserted into the region of its genome corresponding to the US3 gene, in such a way so as to be able to be expressed.

FETHERSTONHAUGH & CO.
OTTAWA, CANADA

PATENT AGENTS

Figure 1/3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digestion KPN 1
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KPN1
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NCO 1
pMDV 52
4947bp
SAC 1
STOP
KPN 1
ATG
mutagenesis
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4947bp
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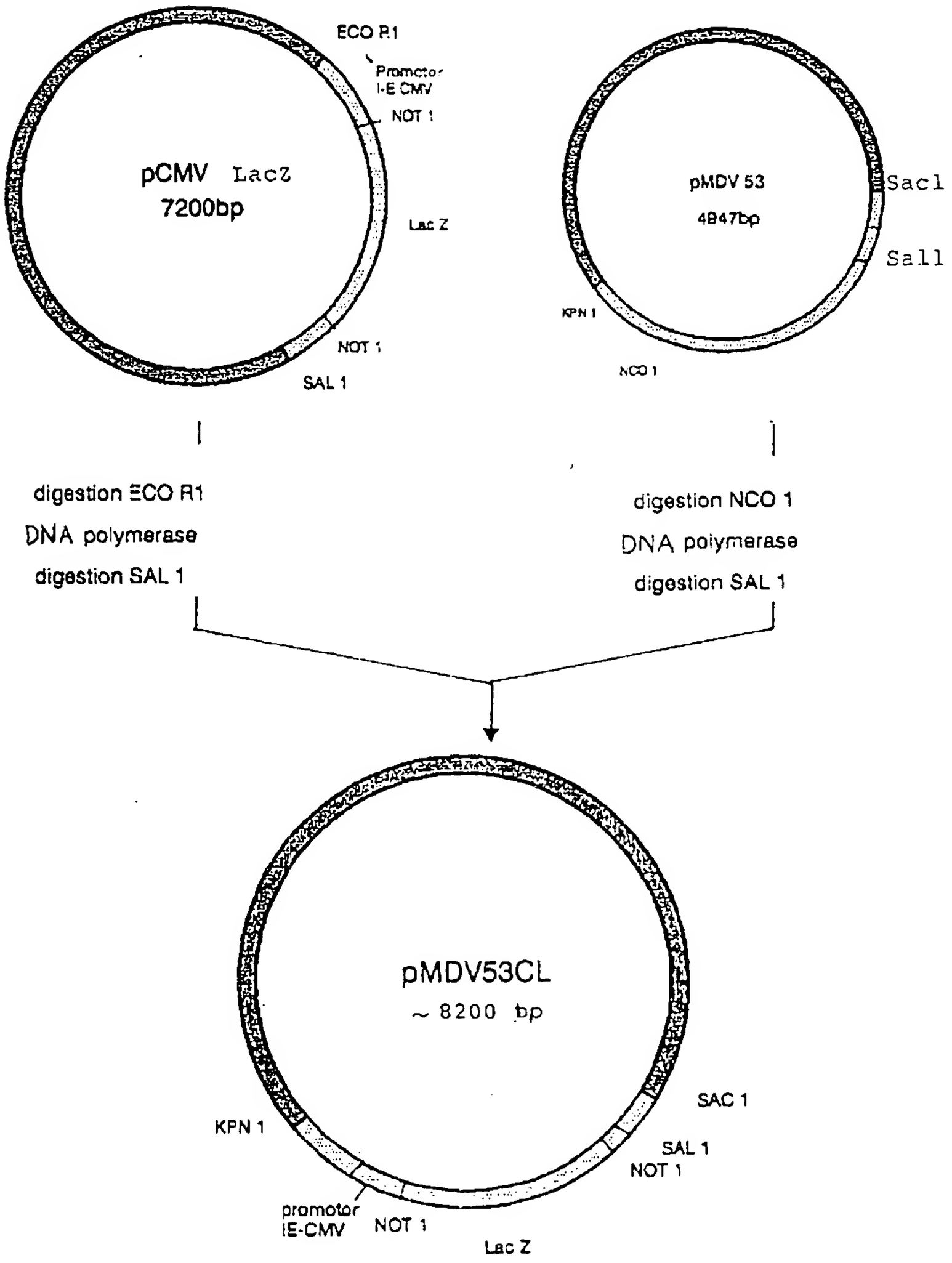
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KPN 1
NCO 1
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digestion NCO 1
DNA polymerase
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promotor
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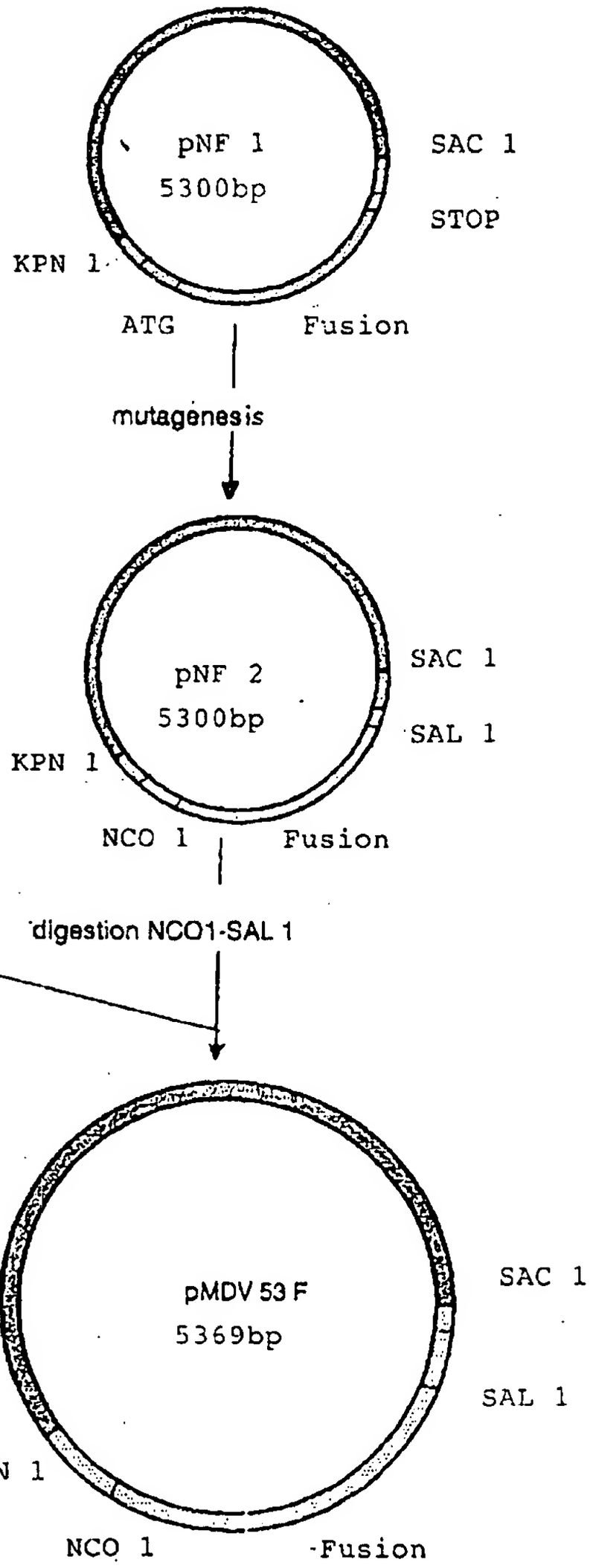
Lac Z

Figure 3/3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